CORRECTED VERSION OF THE CLAIMS

1. (Thrice Amended) A reversible physiological process for temporal separation of oxygen evolution to avoid deactivation of hydrogenase in the presence of oxygen and sustain photosynthetic hydrogen production in cells of an algae microorganism, comprising:

(a) growing a culture of cells of algae microorganism in a medium under illuminated conditions to accumulate an endogenous substrate;

(b) depleting a nutrient selected from the group consisting of sulfur, iron, and/or manganese from the medium until the culture of cells of algae microorganism becomes anaerobic and sealing the culture from atmospheric oxygen;

(c) measuring the rate of cellular respiration of a sample of cells of the algae microorganism from step (b) in the dark with an O_2 electrode;

(d) incubating a sample of the algae microorganism from step (c) in light of saturating intensity of yellow actinic excitation, and measuring the light-saturated rate of O_2 evolution with an O_2 electrode;

(e) inducing reversible hydrogenase through photosynthesis by controlling the light saturated rate of oxygen production from the culture of cells of algae microorganism so that it is equal to or less than a rate of cellular respiration to generate an evolved gas that includes hydrogen.

2. (Amended) The process of claim 1 wherein said hydrogen is generated from water and the accumulated endogenous substrate.

REMARKS

The Official Action and the cited references have been carefully reviewed. The review indicates that the claims, particularly as amended, recite patentable subject matter and should be allowed. Reconsideration and allowance are therefore respectfully requested.

Before addressing the grounds upon which the rejections have been made, applicants' attorney would refer the Examiner to the telephone interview held on October 22, 2002 so as to avoid repeating those understandings that were reached. During the interview, applicants' attorney related that the invention process is very straight forward in that, at the minimum, it entails five steps, as stated on page 3, lines 10-16 of the specification. They are:

growing a culture of the microorganism in medium under illuminated conditions to accumulate an endoglucanase substrate;

depleting from the medium a nutrient selected from the group consisting of sulfur, iron and/or manganese;

sealing the culture from atmospheric oxygen;

incubating the culture in light whereby a rate of light-induced oxygen production is equal to or less than a rate of respiration; and

collecting an evolved gas.

Nevertheless, during the interview, the Examiner required further insertion of steps into the claims to recite the measuring steps needed to ensure that the rate of light-induced oxygen production is equal to or less than the rate of respiration, since it was the Examiner's

conviction that this was all that was needed to place the application in condition for allowance.

Surprisingly, the extensive 14 page Office Action suggests a reversal of the Examiner's belief that the issues had been narrowed to the matter of amending the claims to recite the measuring step so as to avoid the references.

In summarizing the essentials of the invention process, applicants would again relate that its process continuously produces hydrogen by inducing reversible hydrogenase in a manner that provides activity of photosynthesis from a light saturated rate of oxygen production equal to or less than a rate of cellular respiration of algae microorganism.

This innovation is significant because it is known in the prior art that algae will not produce hydrogen gas when oxygen is present because the hydrogenase enzyme that releases hydrogen is not synthesized and is not stable when oxygen is present, wherein the normal plant/algae photosynthetic process splits water and produces oxygen as a by-product, and wherein to get algae to induce the hydrogenase enzyme it has been necessary to use physical (i.e., inert gas bubbling) or chemical (addition of strong reducing agents or biochemical, oxygen-scrubbing systems) means to get rid of the oxygen. Applicants have solved this problem by its discovery of a metabolic switch, whereby removing sulfate from the medium of healthy growing algae rapidly decreases the innate ability of the algae to split water and produce oxygen to only about 10% of their normal ability over a 15 to 30 hour period of time. In this sulfate removal process, applicants have further discovered that algae respiration can take up oxygen at about the level or a little greater (rate)than the cells can produce oxygen (at the lower level of production ability) under sulfur-deprived conditions, and that the culture under these conditions will metabolize all the remaining oxygen in the culture

medium, and the system will become anaerobic. Hydrogen is produced under these conditions because the hydrogenase enzyme is induced by the cells, and this enzyme is stable in the absence of oxygen.

The invention process of providing a reversible physiological process to avoid deactivation of hydrogenase in the presence of oxygen and sustained photosynthetic hydrogen production has unexpectantly been accomplished by:

(a) growing a culture of cells of algae microorganism in a medium under illuminated conditions to accumulate an endogenous substrate;

(b) depleting a nutrient selected from the group consisting of sulfur, iron, and/or manganese from the medium until the culture of cells of algae microorganism becomes anaerobic and sealing the culture from atmospheric oxygen;

(c) measuring the rate of cellular respiration of a sample of cells of the algae microorganism from step (b) in the dark with an O_2 electrode;

(d) incubating a sample of the algae microorganism from step (c) in light of saturating intensity of yellow actinic excitation, and measuring the light-saturated rate of O_2 evolution with an O_2 electrode;

(e) inducing reversible hydrogenase through photosynthesis by controlling the light saturated rate of oxygen production from the culture of cells of algae microorganism so that it is equal to or less than a rate of cellular respiration to generate an evolved gas that includes hydrogen.

Applicants note with appreciation, the withdrawal of U.S. Patent 4,442,211 to Greenbaum consistent with the understanding reached during the interview, for the reason that Greenbaum '211 fails to anticipate applicants' steps of a measuring and controlling the rates of

oxygen production and respiration as required by the claims. In this connection, applicants would also point out that the remaining references are similarly deficient in this regard.

Applicants' representative further notes with appreciation the withdrawal of the Melis et al. publication due to the fact that it is not available against the present invention as applicants' own publication in light of the Affidavit submitted under 37 CFR §1.131.

Claims 1-3, 5-8 and 10 were rejected as being unpatentable over U.S. Patent 4,442,211 and U.S. Patent 4,010,076 taken with Wykoff et al. under 35 USC 103(a).

Applicants respectfully traverse this rejection and request reconsideration for reasons hereinafter provided.

Greenbaum '211 only discloses producing H_2 and O_2 by use of algae in light comprising:

1) subjecting algae in an aqueous phase to light in an environment free of CO_2 and atmospheric O_2 to produce H_2 and O_2 by the action of the light-stimulated algae in splitting water molecules during a first period of time of sufficient duration to produce a physiological stress on said algae;

2) culturing the algae in culture medium in an aerobic atmosphere during a second period of time sufficient to remove the physiological stress; and

3) subjecting the algae in an aqueous phase to light in an environment free of CO_2 and atmospheric O_2 during a third period of time at an enhanced rate of production of H_2 and O_2 compared to that occurring during the first time period of step 1).

Clearly, Greenbaum '211 lacks applicants' step (b) of depleting sulfur until the culture becomes substantially anaerobic.

? S present as trace element and depleted during growth

Additionally, Greenbaum '211 also lacks applicants' steps (c),(d) and (e) which includes inducing reversible hydrogenase through photosynthesis by controlling the light saturated rate of oxygen production so that it is equal to or less than the rate of cellular respiration.

Weetall '076 only disclose a method of continuous photometabolic production of a useful product, comprising immobilizing whole cells of a photometabolically active organism on a medium to form a stabilized composite, supportably placing the composite within a reactor having at least one light transmitting wall, and, in the presence of light being transmitted through the wall, continuously passing into the reactor a substance capable of being photometabolized by the cells under conditions sufficient to assure the production of a useful product.

While blue-green algae may be used in the biophotolysis of water by oxidizing the water and reducing NADP to NADPH, the combination of Weetall '076 with Greenbaum '211 is deficient insofar as suggesting or teaching applicants' steps (b) and (d), or depletion of the sulfur nutrient and incubating the culture in light to induce reversible hydrogenase to provide activity of photosynthesis from a light saturated rate of oxygen production, equal to or less than the rate of cellular respiration.

The deficiencies discussed above in reference to Greenbaum '211 and Weetall '076 are not compensated for by any teachings in the secondary reference of Wykoff et al.

Wykoff et al. only disclose the extent to which the light-saturated rate of photosynthetic O_2 evolution declines in *Chlamydomonas reinhardtii* upon P and S starvation. It makes no reference to or acknowledgement of, the prior art problem of not being able to sustain hydrogen production due to deactivation of hydrogenase in the presence of oxygen during photosynthetic hydrogen production. Neither does Wykoff et al. provide any solution to this problem.

Accordingly, even if Wykoff et al. were combined with Greenbaum '211 and Wetall '076, applicants' claims as presently amended would clearly not result.

Neither would applicants' process be rendered obvious for the reason that no reference alone or in combination either acknowledge or resolve the problem of providing sustained production of hydrogen by avoiding deactivation of hydrogenase in presence of oxygen by controlling the light saturated rate of oxygen production so that it is equal to or less than the rate of cellular respiration.

Even if the Wykoff et al. teachings of the extent to which the light saturated rate of photosynthetic O_2 evolution declines in *Chlamydomonas reinhardtii* upon P and S starvation were substituted into the processes of the primary references of Greenbaum '211 and Wetall '076, such a substitution would be inadequate without hindsight reference to applicants' invention, to provide a skilled person in the art with means for sustaining production of hydrogen by avoiding deactivation of hydrogenase in the presence of oxygen, as required by applicants' claims, as presently amended.

The examiner is again reminded of her acknowledgement in the interview of 10/22/02 that there was allowable subject matter, provided the claims are amended to recite the measuring steps needed to ensure that the rate of light-induced oxygen production is equal to or less than the rate of respiration.

Note is taken of the objections raised to claims 1-3, 5-8 and 10, the rejection of claims 1-3, 5-8 and 10 under the second paragraph of 35 USC §112 on allegations of indefiniteness and the rejection of claims 1-3, 5-8 and 10 under the first paragraph of 35 USC §112 on allegations of new matter; however, in view of the amendments made to the claims, these objections and rejections are believed no longer applicable.

In view of the foregoing amendments, remarks and arguments, it is believed that the application is now in condition for allowance and early notification of the same is earnestly solicited.

Respectfully submitted,

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Paul J. White
Attorney for Applicants
Registration No. 30,436

NATIONAL RENEWABLE ENERGY LABORATORY
1617 Cole Boulevard
Golden, Colorado 80401-3393
Telephone: (303) 384-7575
Facsimile: (303) 384-7499

CERTIFICATE OF MAILING UNDER 37 CFR § 1.8

I hereby certify that the following attached item Amendment under 37 C.F.R. § 1.115 is being deposited in the United States Postal Service as first class mail, postage pre-paid, in an envelope addressed to: Assistant Commissioner for Patents, U.S. Patent & Trademark Office, P. O. Box 1450, MS Non-Fee Amendment, Alexandria, VA 22313-1450 on this 16th day of May 2003.

Brenda E. Brantley
Senior Patent Administrator